ESRP: MEASURING THE RESPONSE OF SOIL MICROBIAL COMMUNITIES TO CHEMICALS IN PESTICIDES AND HERBICIDES USING 16S rRNA SEQUENCING

Lincoln-Way East High School ESRP Team 2024

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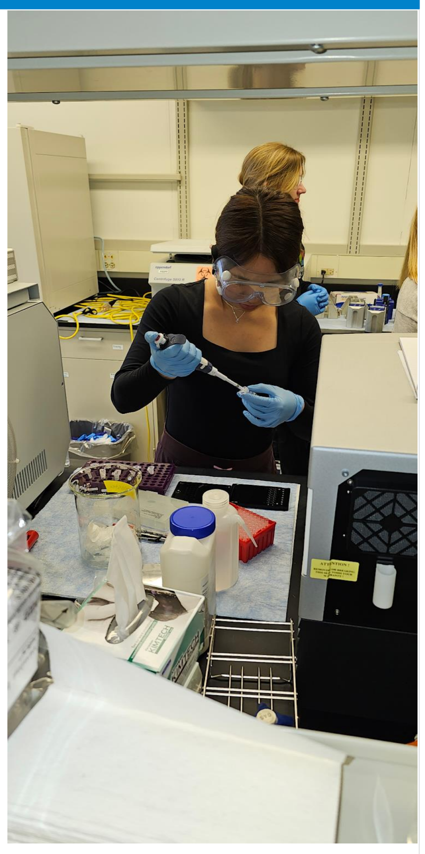
ABSTRACT

Alterations in the chemical composition of soil have a direct impact on its biodiversity. Recognizing the impact of chemicals, such as those in pesticides, on soil microbes is important to understanding chemical effects in the environment. In our project, we identify relationships between soil biodiversity and the application of various stressors. Since chemicals are used frequently in modern agriculture, knowing their effects on bacteria will help develop a deeper understanding of the human impacts on the environment. We exposed soil samples to five commonly used chemicals (Gamma Cyhalothrin, Malathion, Atrazine, Fipronil, and Urea) in varying concentrations and exposure times. After the samples were aged appropriately, we used 16S sequencing to quantify their bacterial diversities. Using R, we modeled exposure time, chemical type, and chemical concentration against bacterial concentrations resolved from the 16S sequencing. These models enable us to draw conclusions on the overall impact of pesticide, herbicides, and fertilizer use on bacterial diversity.

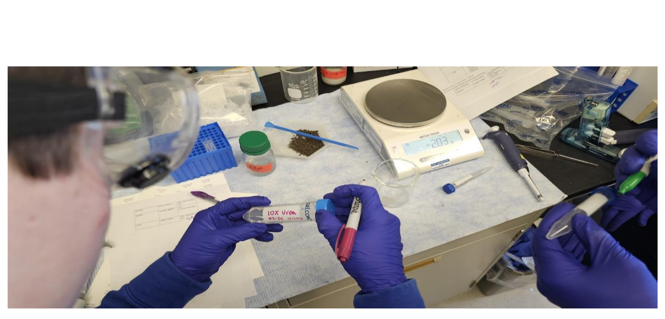
MOTIVATION



Our project mentors, Sara Forrester and Justin Podowski, worked on a paper titled NA22. NA22 was a research experiment focused primarily on the impact of PUREX (plutonium uranium reduction extraction) chemicals and how they affected the microbiomes of soil. Our project builds upon that previous experience, shifting the focus from PUREX chemicals to the impact of pesticides and other common chemicals on soil diversity. By examining the effects of garden pesticide stressors on soil microbial communities, we aim to uncover the potential risks these chemicals pose to soil and crop microbiomes. Through our investigations, we aim to contribute to a deeper understanding of how chemical pollutants influence soil ecosystems and bacterial growth dynamics.

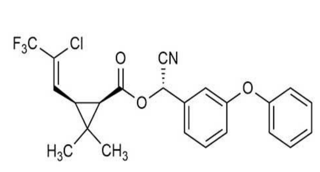
METHODS (SAMPLING AND DNA EXTRACTION)

- The 100 samples created began with soil from FermiLab near Chicago. This soil was sifted through, and any seeds, sticks, and other non-soil debris were sorted out.
- Then, solutions of the chemicals at different concentrations (.1, 1.0 and 10X) were created by dilution. All fertilizers were liquid except for urea, which was dissolved into a solution before being diluted.
- Every petri sample dish had equal grams of soil and received the same volume of a solution-either chemical or negative control (water).
- 5 chemicals tested x 3 molarities of each x 6 soil samples for each chemical and molarity + 10 negative controls = 100 total samples
- The DNeasy PowerSoil Pro Kit was used to extract DNA. This kit's chemicals and instructions result in pure DNA that can be analyzed by comparing genetic markers to a microorganism database.
- The experimental soils were prepared with a solution and beads that separate the DNA from the nucleus. This solution was centrifuged to separate the soil from the microbial components. The microbes' cell parts were separated and underwent additional centrifuging and chemicals to separate the DNA from the other cell components, such as organelles.
- The now-separated DNA was stored and used for analysis.



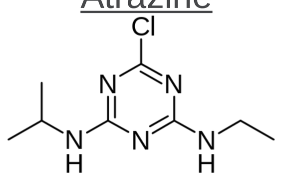
THE 5 CHEMICALS

Gamma Cyhalothrin



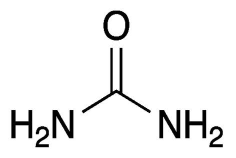
- Chemical formula: C23H19ClF3NO3
- Synthetic organic insecticide
- High efficiency and low toxicity when compared to organophosphates

Atrazine



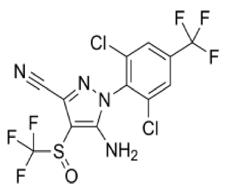
- Chemical formula: C8H14ClN5
- Herbicide often mixed with carrier to kill weeds and regulate plant growth
- Works to block Chloroplast electron flow: limiting photosynthesis and killing the plant

Urea



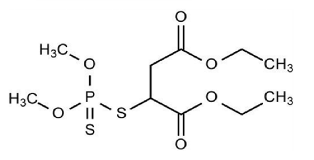
- Chemical formula: CO(NH2)2
- Amide used in the agricultural industry as one of the most nitrogenous fertilizers
- Quickly converts nitrogen into ammonium bicarbonate

Fipronil



- Chemical formula: C12H4Cl2F6N4OS
- Phenylpyrazole insecticide; organophosphate
- Slow acting with the purpose of expanding effects to nests and colonies

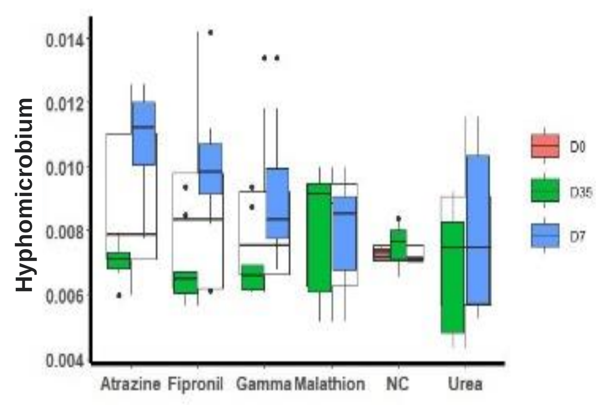
Malathion

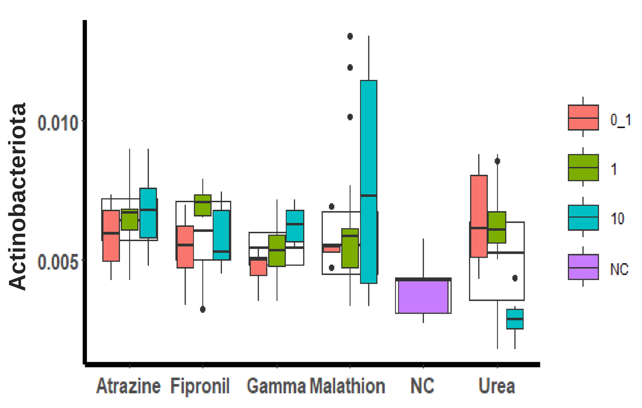


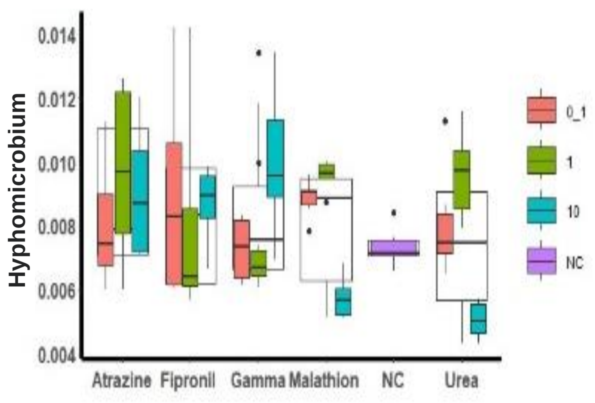
- Chemical formula: C10H19O6PS2
- Organophosphate insecticide used in agriculture
- Works to paralyze and suffocate insects

DATA ORGANIZATION AND ANALYSIS

Malathion

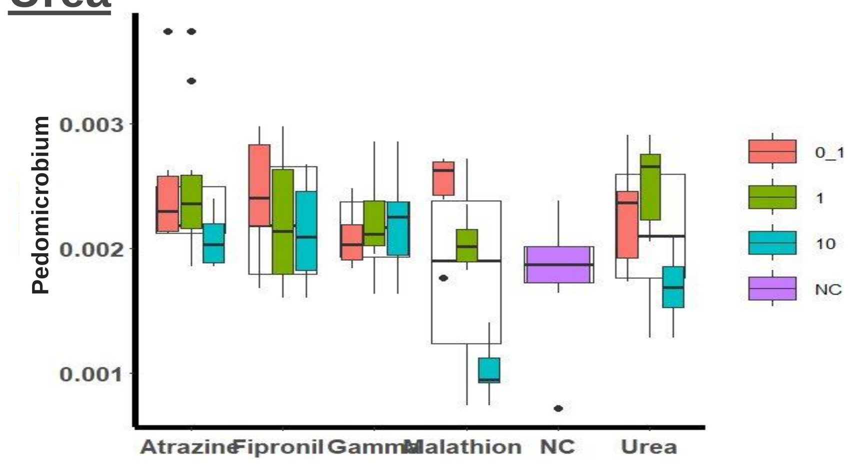


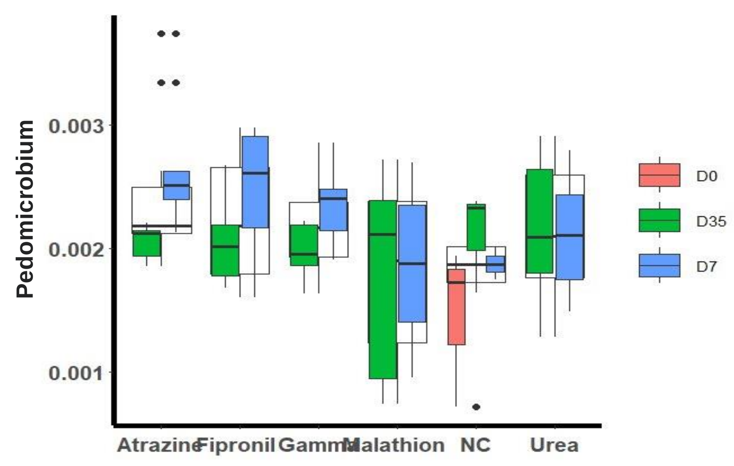




The half-life of Malathion in soil, water, and plants is known to be less than 1 week; because of this high degradation rate, Malathion appears to have no effect on the concentration of the various bacteria in the soil after the first week. Increased concentrations of Malathion lead to lower productions of CO2; CO2 is often crucial to bacterial life within the soil; when deprived of this CO2 due to increased quantities of the insecticide Malathion, the bacterial concentration show a decrease (as shown by ASV6, Hyphomicrobium). Since bacteria within the phylum of Actinobacteriota (Ex: ASV15) can tolerate conditions with low CO2 levels, they showed no decrease when exposed to Malathion.

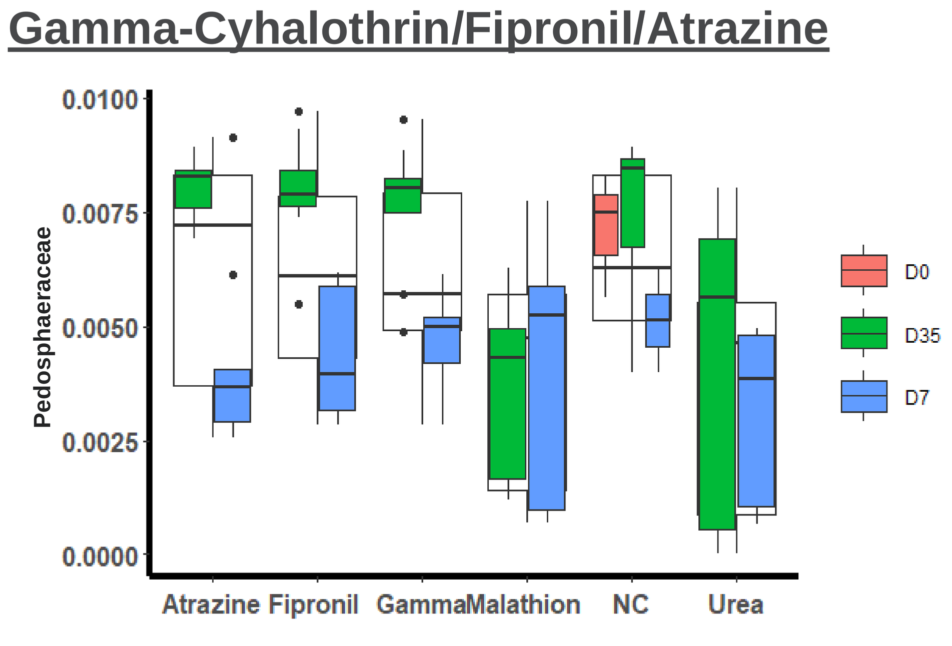
Urea

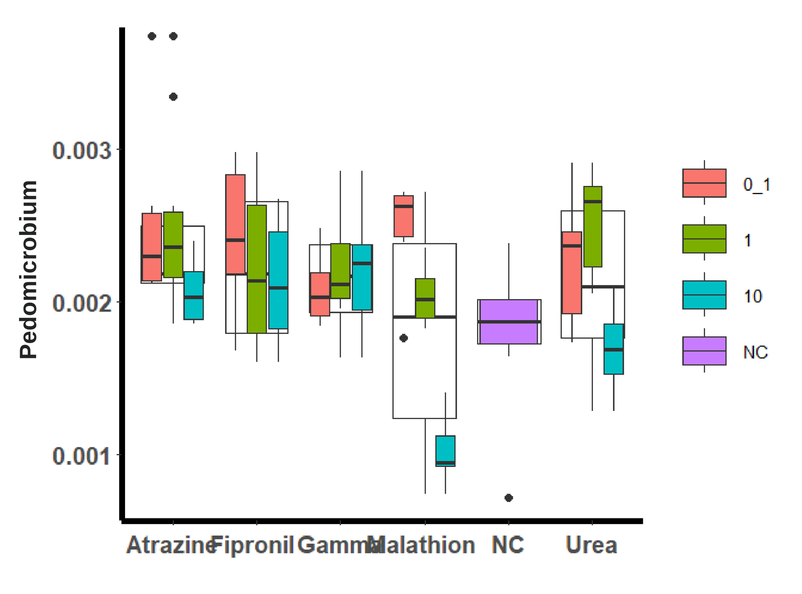




In the soil, urea transforms into ammonium bicarbonate through hydrolysis, which causes a temporarily high pH. This can be harmful to the bacteria and decrease the bacterial concentration. Pedomicrobium's order is rhizobiales, which consists of nitrogen-fixing bacteria; therefore, bacteria concentration is unchanging because enough nitrogen has already been fixed and pH is ideal. This can be observed in the two graphs, in both cases concentration of the respective bacteria remain relatively constant regarding urea. Pedomicrobium is specifically known to oxidize Manganese, so the concentration of the bacteria may also be unchanging since Urea is not involved with Manganese.

Gamma-Cyhalothrin/Fipronil/Atrazine





All three have similar equilibrium conditions, which can be because of degradation and shared pesticide qualities. Organic matter content, soil texture, and pH influence their similar sorption. Also, Atrazine and Fipronil are known as Class II moderately hazardous pesticides according to WHO, explaining the similar patterns found in the difference of microbiome diversity. Bacteria concentration significantly decreases for 10x concentration of these two since these chemicals can lead to varying degrees of toxicity, which suggest harm to the nitrogen-fixing bacteria. 10x Gamma-Cyhalothrin, however, increases the concentration of pedomicrobium because it is less toxic than a Class II moderately hazardous pesticides.

Note: ASV stands for Amplicon Sequencing Variant. ASVs represent different bacteria that we identify in our microbial communities

MAJOR ACCOMPLISHMENTS

-Created 100 soil samples featuring our chemicals at various concentrations

-Successfully extracted DNA from our soil samples using the DNeasy PowerSoil ProKit

-Used 16S rRNA Sequencing to determine our chemicals' genetic variation

-Learned how to utilize RStudio to construct models of ASVs over different periods of time and at varying chemical concentrations

-Found patterns in our bar graph models to analyze the underlying microbial functions and significance of our chemicals

FUTURE DIRECTIONS

- **Data Bank:** Our findings regarding the bacterial concentrations in respect to chemicals of varying concentrations will contribute to future data banks. These data banks will contain information relative to bacterial patterns to help identify unknown chemicals in soil samples. By identifying which chemicals have been in soil samples, it will simplify the method in which people are held accountable for what is put into the environment.
- **Agriculture:** To ensure the long-term stability of agricultural cultivation with the growing demand for food and biofuels, our experiment can be used to answer how the presence of chemicals in pesticides affects the health of the soil and the resulting productivity of crops. Understanding these effects can lead us to find strategies to mitigate the risks associated with chemicals found in agricultural systems.
- **Pollution:** Because pesticides tend to have far-reaching impacts beyond application sites due to run-off, our analysis can be utilized to investigate how far the chemicals are potentially being leached and transported into groundwater and other surface bodies of water. We can determine if there are any traces of pesticides in the area by analyzing bacterial patterns.

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